RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Quantitative Autoradiography with Radiopharmaceuticals, Part 1: Digital Film-Analysis System by Videodensitometry: Concise Communication

Yoshiharu Yonekura, A. Bertrand Brill, Prantika Som, Gerald W. Bennett, and I. Fand

Brookhaven National Laboratory, Upton, New York, and State University of New York at Stony Brook, Stony Brook, New York

A simple low-cost digital film-analysis system using videodensitometry was developed to quantitate autoradiograms. It is based on a TV-film analysis system coupled to a minicomputer. Digital sampling of transmitted light intensities through the autoradiogram is performed with 8-bit gray levels according to the selected array size (128 × 128 to 1024 × 1024). The performance characteristics of the system provide sufficient stability, uniformity, linearity, and intensity response for use in quantitative analysis. Digital images of the autoradiograms are converted to radioactivity content, pixel by pixel, using step-wedge standards. This type of low-cost system can be installed on conventional mini-computers commonly used in modern nuclear medical facilities. Quantitative digital autoradiography can play an important role, with applications stretching from dosimetry calculations of radiopharmaceuticals to metabolic studies in conjunction with positron-emission tomography.

J Nucl Med 24: 231–237, 1983

Since the method for whole-body autoradiography (ARG) was established by Ullberg (*1*), many biomedical applications of ARG have been developed, especially in the field of pharmacology and toxicology (*2,3*). Recently, in nuclear medicine, many new gamma- and positron-emitting radiopharmaceuticals have been introduced, and their distribution and metabolism in normal and diseased states need to be evaluated. The use of ARG provides the high spatial resolution required to determine radiopharmaceutical biodistribution in small animals. When quantitative information is extracted, ARG can play more important roles, increasing our understanding of the function and metabolism underlying different disease states (*4*).

The present paper describes a simple, low-cost system for digital film analysis of ARG. It is based on videodensitometry (*5*), and uses a TV camera coupled to a minicomputer. Quantitative digital ARG methods have been developed using this system, not only for ARG with beta emitters, but also for gamma- and positron-emitting nuclides (*6*).

Received Aug. 10, 1982; revision accepted Nov. 1, 1982.

For reprints contact: A. B. Brill, MD, PhD, Medical Department, Brookhaven National Laboratory, Upton, New York 11973.

MATERIALS AND METHODS

Videodensitometry system. A schema of the system is shown in Fig. 1. A chalnicon TV camera (Model C-1000-01)* is coupled—via a camera control unit (M999-05)*, an A-D converter, and a DMA interface (DR11B)—to a minicomputer. The video signal from the camera is digitized column by column. Hence, when one digitizes an image into 128 × 128 elements, the time required is ~4 sec (i.e. 128 columns/frame × 1/30 sec/frame). The data are digitized into 256 levels (8 bits) and the digitized data transferred to the computer through the DMA interface. These data are archived on a disk storage system or a magnetic tape, and displayed on a color monitor or black and white monitor. An adaptor ring permits the camera to be used with standard

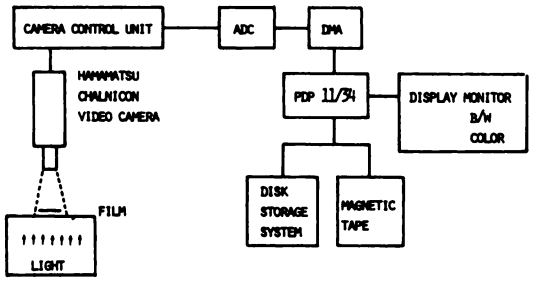

FIG. 1. Schematic diagram of TV-film analysis system. Video signals of light intensities transmitted through ARG films are digitized by ADC, transferred to PDP 11/34A through DMA interface, and archived in a magnetic-disk storage system.

photographic lenses. By proper choice of lens and distance, the size of the field of view can be adjusted for almost any desired film size. The camera control unit provides gain controls for the TV camera, low-level bias adjustment, and variable digitizer mesh size (256 × 256, 512 × 512, and 1024 × 1024). The light box constructed for this application consists of eight tungsten-filament light bulbs, in a rheostat-controlled box covered with ground glass (30 × 40 cm).

The transmitted light intensities through the ARG films are digitized with 8-bit gray levels according to the selected array size (128 × 128 to 1024 × 1024). The maximum spatial resolution is obtained by digitizing an area 1 × 1 cm with a 1024 × 1024 array using a close-up photographic lens. In this case, the pixel size is 10 × 10 μm. These data are stored on a 5-megabyte disk for further analysis.

The performance characteristics of the system were examined for uniformity and linearity of the field, spatial resolution, effect of signal averaging, and the light vs. intensity response. The overall uniformity of the system was studied with different light intensities. The linearity and the spatial resolution were tested with the reflection and transmission images of linear grids and a TV test pattern (*7*). Calibrated step-wedge films were used to measure the response characteristics over a wide range of light intensities. The optical density of the step-wedge films was also measured with a manual densitometer, and these values were compared with the digitized numbers obtained by videodensitometry.

Quantitative digital autoradiography. Whole-body autoradiograms of mice were prepared using a cryomicrotome as described in Part 2 of this paper (*6*). Commercial C-14 methylmethacrylate standards (0.022–9.7 μCi/g), calibrated to the ^{14}C-2-deoxy-D-glucose (C-14 DG) activity of 20μ-thick brain sections, were used for C-14 autoradiograms. For the autoradiograms of gamma- and positron-emitting compounds, filter papers were soaked in serial dilutions of the compounds. The dried step-wedge standards made from them were counted in a well scintillation counter and placed in contact with the film adjacent to the tissue sections being studied. Major organs showing relatively uniform distribution, such as liver, heart, blood pool, and muscle,

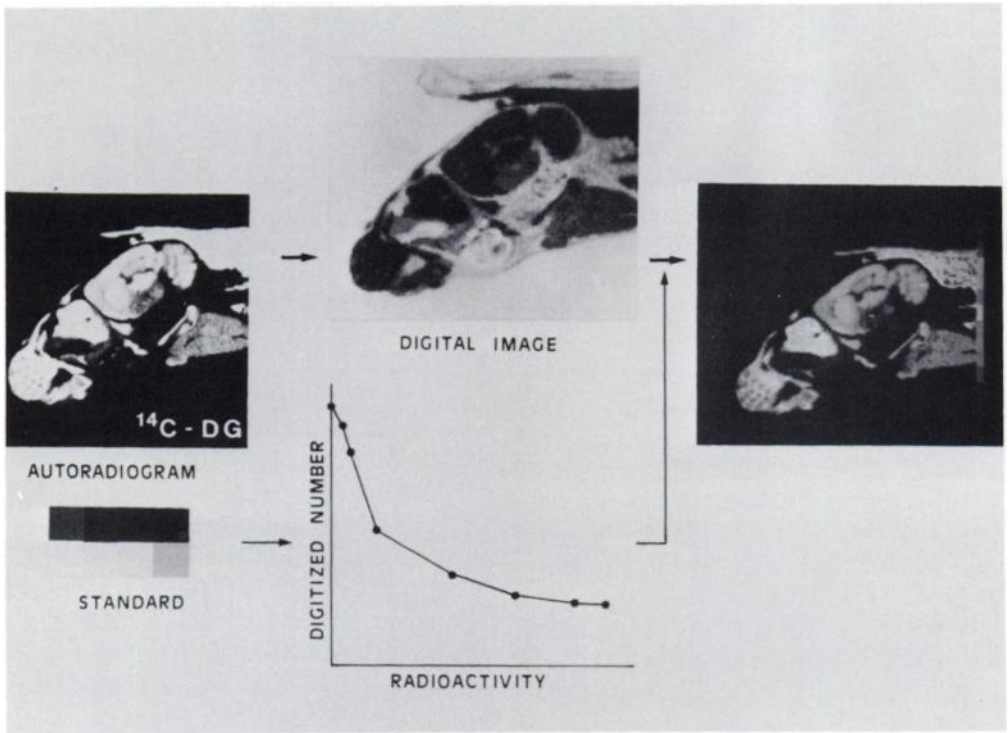

FIG. 2. Method of quantitative digital autoradiography. Films of ARG and standards are digitized at same light intensity. Digital image of ARG (middle upper image) was converted to a map of radioactivity (right image–original in color) using the standard curve (middle lower image).

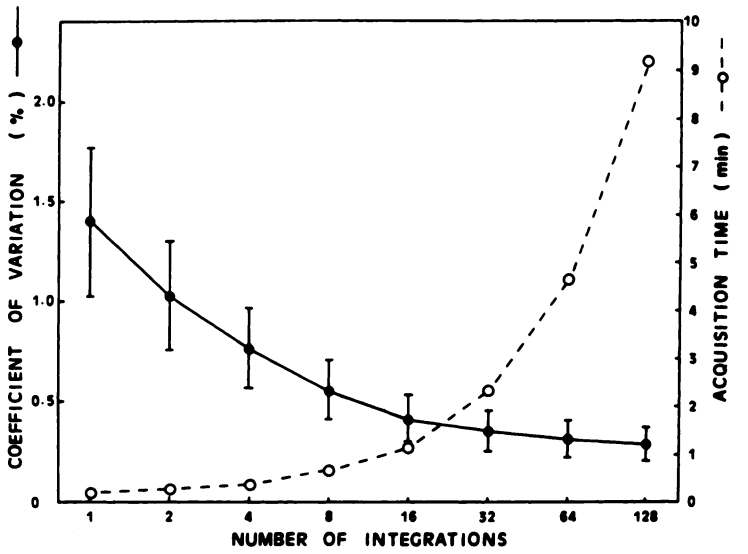

FIG. 3. Effect of signal averaging with multiple integrations. Image was digitized in an 128 × 128 array, and values of 9 pixels in center of image were used for calculation. Corresponding values for coefficient of variation (solid circle) and data acquisition time (open circle) are shown.

were taken for counting from the tissue block left after sections had been cut. These measured activities were used for final calibration to obtain the absolute values of activity in autoradiograms with gamma- and positron-emitting compounds.

Autoradiographic films were put on the light box, and transmitted light intensities through these films were digitized in a darkened room. The standard curve obtained from the digitized images of the step-wedge standards was used to convert the digital images of ARG to the radioactivity content, pixel by pixel (Fig. 2). This procedure was performed by linear interpolation between adjacent calibrated values.

Digitized data are stored in the computer's memory and disk system as 128 × 128 to 1024 × 1024 arrays. Since the display system is limited to 128 × 128 arrays, data from larger arrays are either compressed to 128 × 128 by the summing of adjacent pixels, or multiple 128 × 128 array images are composited to create images with larger array sizes. In addition to the routine software being used for data processing in nuclear medicine, some special application programs have been installed. These include magnified display of small regions with 128 × 128 array from the data obtained with high resolution, display enhancement with window setting or histogram equalization (*8*), and calculation of means and standard deviations of activities in the pixels within regions of interest determined by joystick control. These utility programs are written in FORTRAN.

RESULTS

Performance characteristics of the system. Since one of the major problems of TV measurement systems is noise sensitivity, signal averaging is necessary to improve the signal-to-noise ratio. Figure 3 shows the effect of signal averaging with increasing numbers of integrations. Signal averaging involving 16 integrations was found to be sufficient for stable digital sampling (cv <0.5%). The uniformity measured over the entire light-box surface revealed a coefficient of variation less than 2% with 16 integrations. Measured distortion of spatial linearity is less than 1% with a standard photographic close-up lens. The spatial resolution of the system depends on the focal length of the lens, the lens-to-film distance, and the array

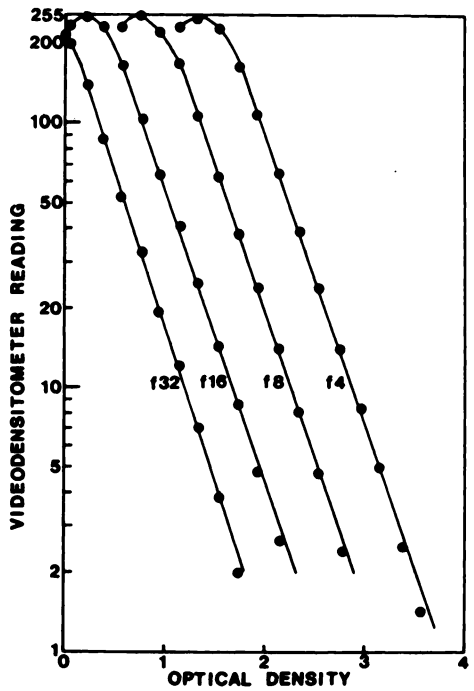

FIG. 4. Relation between optical density and digitized light intensity with different lens openings.

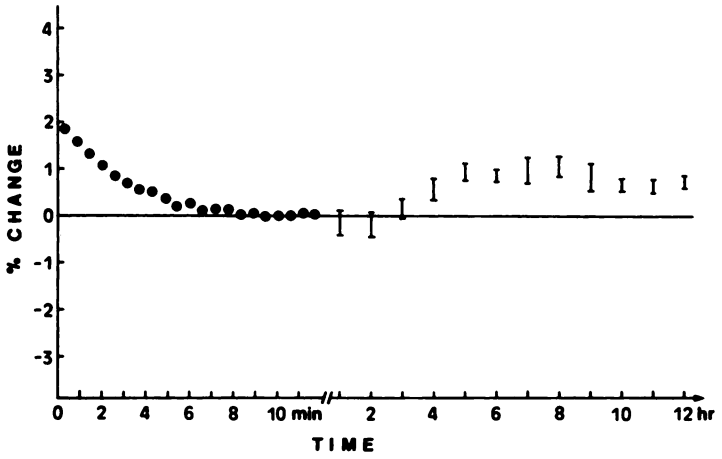

FIG. 5. Stability of system. Light intensity in center of field of view was digitized for 12 hr. Values are shown as percent change from that at 10 min after initially turning on the system. Maximum range within every hour is shown for first 12 hr.

size used. The digitized images of a standard TV test pattern showed that the system can resolve 500 TV lines along the vertical height and 400 TV lines along the horizontal dimension of the field when a suitably large array size is used. When a field of 1 × 1 cm is digitized into a 1024 × 1024 array, 50 lines per mm can be resolved; the pixel size is then 10 × 10 μm.

Figure 4 shows the relation between optical density and digitized light intensity measured from a calibrated step-wedge film at a selected intensity of the light source. The response is linear for a broad range of optical densities when different lens openings are used. The response characteristics of the system rarely require digitization with more than a single lens opening. Ordinarily an analysis is performed with F/8 to F/22, and the intensity of the light source and the gain controls of TV camera are adjusted to use the linear part of the response curves.

A usual procedure involves 16 integrations to reduce noise from the video camera system. This requires 70 sec for acquiring and signal-averaging a 128 × 128 array image. Figure 5 shows the stability of the system as assessed by repeatedly digitizing the output of the light box for 12 hr. After initially turning on the system, it took approximately 10 min for a stable value to be recorded. Short-term transients were not seen. Long-term drifts, which ranged up to 2%, were observed over several hours. Since the procedure involves reference to calibration standards with each image processed, these slow drifts do not interfere with the accuracy or precision of the study analyses.

Quantitated digital images of ARG. The whole-body ARG of a mouse given C-14 DG was digitized as four consecutive images. The quantitated digital image is shown in Fig. 6, along with the original ARG image. The high spatial resolution of the photographic image appears to be preserved in these digital images. Activities of major organs obtained from quantitative digital ARG in different sections are shown in Table 1. Variations of these values are not large except in skeletal muscles, where considerable regional difference in activity can be seen.

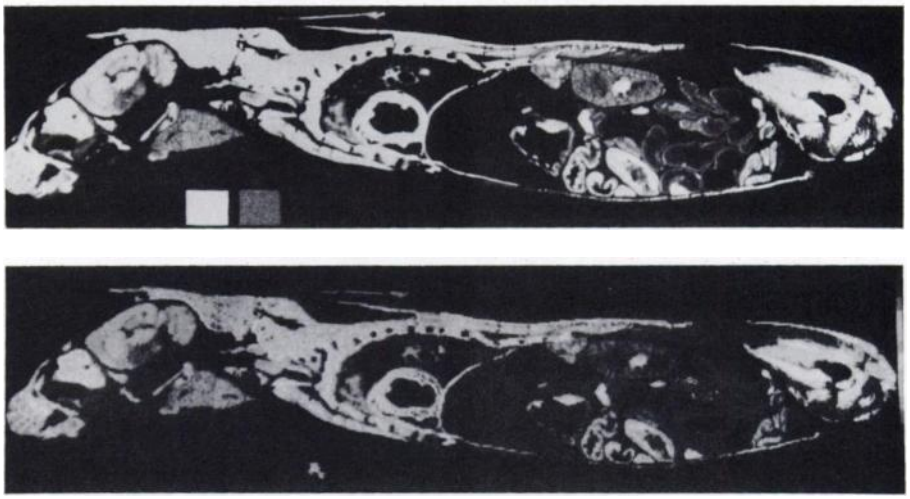

FIG. 6. Original ARG (upper) and quantitative digital ARG (lower–original in color) images of mouse given C-14 DG at 45 min before sacrifice.

TABLE 1. VALUES EXTRACTED FROM QUANTITATIVE DIGITAL ARG

Section	C-14 DG activity (nCi/g)*							
	Harder's gland	Brain	Myocardium	Blood pool	Lung	Liver	Kidney	Muscle
1	389.8	163.4	766.1	23.0	42.9	21.9	—	130.7
2	281.5	146.6	810.9	24.4	52.0	28.0	64.1	184.8
3	333.8	143.5	814.7	27.6	56.3	28.5	—	164.2
4	299.7	139.1	800.2	16.5	52.0	23.6	71.7	133.3
5	—	—	718.0	21.6	44.8	23.6	54.2	192.2
6	—	—	883.8	—	36.0	34.7	77.7	258.0
7	—	—	715.2	—	28.2	30.0	73.8	259.6
Mean	326.2	148.2	787.0	22.6	44.6	27.2	68.3	189.0
±s.d.	47.6	10.6	59.5	4.1	9.9	4.5	9.3	53.1
CV (%)†	14.6	7.2	7.6	18.2	22.2	16.5	13.6	28.1

* The mouse was killed at 45 min after intravenous injection of C-14 DG (2.5 μCi), and the mean value in the organ was calculated in each section of ARG.

† Coefficient of variation.

In case of ARG with gamma- and positron-emitting compounds, the step-wedge standards do not provide absolute values of activity, and final calibration of the values in the digital image is obtained from tissue-counting data. Figure 7 shows a comparison of these tissue-counting data with the final quantitated ARG obtained from the same animal. This procedure provides the absolute values of activity (μCi/g or cpm/g) as well as uptake ratio (% injected dose/gram tissue) in small regions.

The system permits one to digitize any size of image

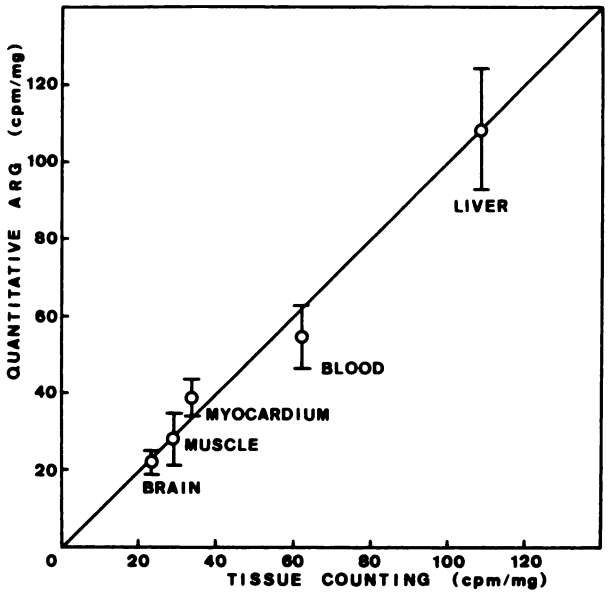

FIG. 7. Comparison of tissue-counting and quantitative ARG in same mouse given [^{131}I]iodoantipyrine at 30 sec before sacrifice. Mean and standard deviation were calculated by averaging values in pixels within regions of interest.

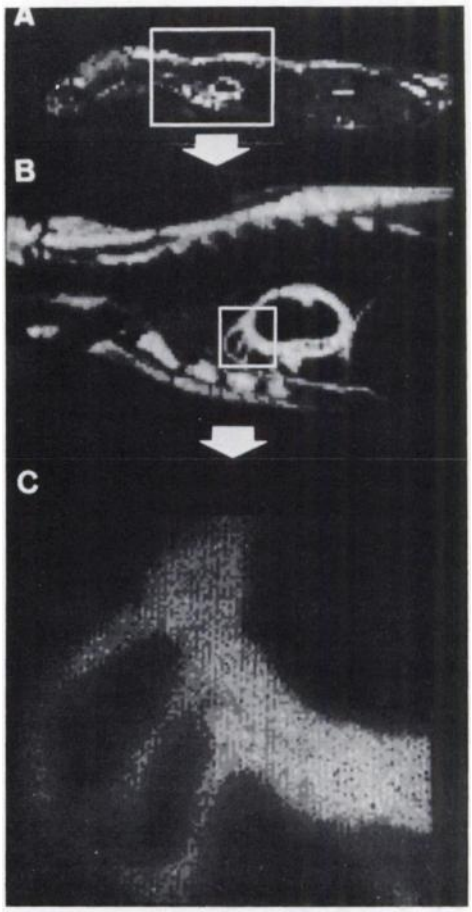

FIG. 8. Digital images of ARG (mouse given C-14 DG) with different magnifications (originals in color). A: Field of 10 × 10 cm was digitized with 128 × 128 array (780 × 780 μm pixel size). B: Field of 2 × 2 cm was digitized with 128 × 128 array (156 × 156 μm pixel size). C: Same field as B was digitized with 1024 × 1024 array (20 × 20 μm pixel size), and only a part of 128 × 128 array was displayed.

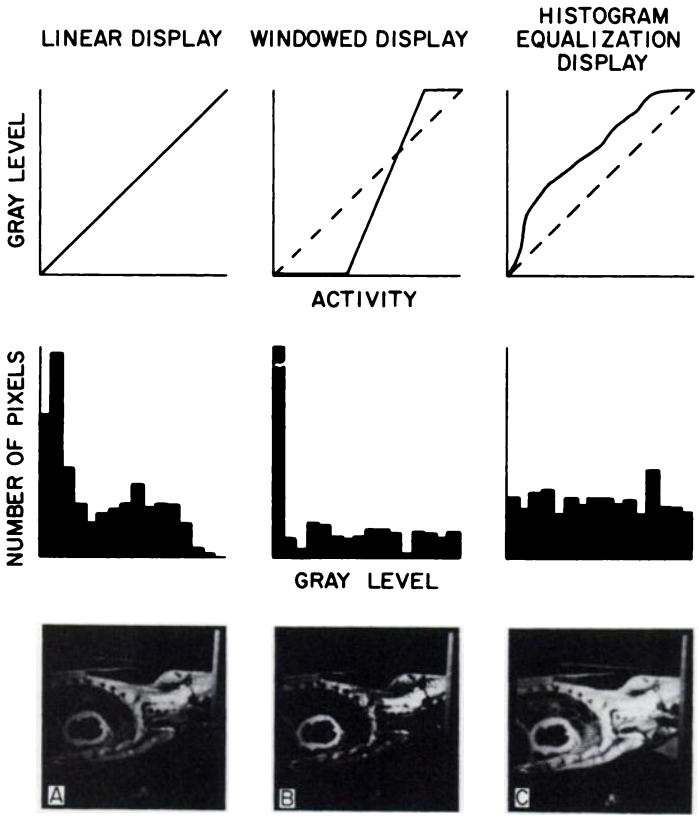

FIG. 9. Examples of enhanced display, with ARG of Fig. 6. Gray level vs. activity relation (top) and number of pixels in each gray level (middle) are plotted schematically for three different displays (bottom). A: Linear gray-scale display. B: Enhanced display with windowing. C: Histogram-equalization display.

ranging from 1 × 1 cm to 30 × 30 cm into 128 × 128 to 1024 × 1024 arrays. Figure 8 demonstrates image magnification by changing the size of the digitizing image field and by changing the array size. Using these strategies, even small structures in mice can be resolved and quantitated. The images are displayed on monochrome and color-TV monitors. The color-coded images reveal more levels than can be perceived on the monochrome displays, whereas the monochrome displays provide an immediately recognized intensity scale that complements the color displays. Since the quantitated digital images of ARG are directly correlated with the activity in the sections, these images usually displayed in linear gray scales. Figure 9 shows the examples of the enhanced displays, which are used frequently. The windowing of intensity regions provides contrast enhancement in the selected range of activity, whereas histogram-equalization displays are used to present images with wide variations in gray scale.

DISCUSSION

Systems for quantitative film analysis have been used successfully in many different high-technology applications (*9*). Development of simple, low-cost systems now permits us to utilize this approach for medical applications. We are using a system based on a TV camera coupled to a computer. The major advantage of this system is that it is relatively inexpensive when installed on a computer that is being used for routine nuclear medical data processing. Moreover, it is possible to use this system for a variety of applications, including analysis of transparent films, as well as dynamic images of reflected light intensities from moving objects viewed by the TV camera. However, the TV-camera system needs to be signal-averaged to improve the signal-to-noise ratio, and this prolongs the data acquisition. Signal averaging involving sixteen integrations of the output signals requires 70 sec for acquisition of a 128 × 128 image, and yields a coefficient of variation less than 0.5%, which is acceptable compared with other systems (*10*) for analysis of static images.

The autoradiographic method provides an important tool for medical research and is especially useful for the development of new radiopharmaceuticals, including assessment of regional biodistribution in small structures and calculations of radiation dosimetry. Using this technique, small accumulations in parts of organs can be seen easily, and the improved sectioning equipment now permits the preparation of whole-body ARGs of animals ranging in size from a small mouse to a large monkey with section thickness of 5 to 100μ.

Semiquantitative analysis of ARGs has been performed by many investigators (*4,11*). In general they used manual densitometers to make several readings over areas of interest, which were then averaged. Recently, Goochee et al. (*10*) have devised a system to quantitate ARGs using a computer-coupled scanning densitometer.

Our TV-based system has comparable capability with less cost. The maximum spatial resolution is obtained with a pixel size of 10 × 10 μm, and it exceeds the resolution required for most macro-ARGs except for those labeled with tritium, a low-energy beta emitter.

Since we are using different types of film for long-lived beta emitters and short-lived gamma- and positron-emitters (*6*), corrections for different film response characteristics are needed. These are measured from standard curves, that are obtained by simultaneous exposure of serial dilution standards with sections on the films. Furthermore, sample counting of parts of organs that are left in the blocks after sectioning provides the absolute values of activity to be compared with the values obtained from the quantitated digital ARG image of the adjacent section. Usually this calibration is performed in relatively large organs with uniform concentration of the tracer, such as liver. This procedure makes it possible to quantitate biodistribution of gamma- and positron-emitting compounds in small regions.

Although the system has the capability to digitize a 1 × 1 cm object into 1024 × 1024 image elements, the spatial resolution is limited by the optical system and the resolution of ARG films. Usually, analysis was performed by digitizing a field of 2 × 2 cm into a 128 × 128 or a 256 × 256 array, where the pixel size is 156 × 156 μm or 78 μm, respectively.

The quantitative ARG method described in this paper can be applied not only for C-14 compounds but also for many gamma- and positron-emitting compounds as well as for tritium or other low-energy beta emitters. It can also provide information on radioreceptor mapping, neurotransmitter, and neuroleptic drug distribution in animals, complementing the capability of current PET imaging systems.

FOOTNOTE

* Hamamatsu Systems, Inc., Waltham, MA.

ACKNOWLEDGMENTS

The authors thank Mr. D. Sacker and Mr. R. Dobert for technical assistance and Mrs. E. Roche for preparation of the manuscript.

Work performed under Contract No. DE-AC02-76CH00016 with the US Department of Energy. By acceptance of this article, the publisher and/or recipient acknowledges the US Government's right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

REFERENCES

1. ULLBERG S: Studies on the distribution and fate of ^{35}S-labeled benzylpenicillin in the body. *Acta Radiol* Suppl 118: 1-110, 1954
2. GAHAN PB, Ed: *Autoradiography for Biologists*, Academic Press, New York, 1972, pp 19-31
3. ROGERS AW: *Techniques of Autoradiography*, Elsevier, Amsterdam/New York/Oxford, 1979, pp 3-11
4. SOKOLOFF L, REIVICH M, KENNEDY C, et al: The (^{14}C) deoxy-glucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28: 897-916, 1977
5. KEDEM D, KEDEM D, SMITH CW, et al: Velocity distribution and blood flow measurements using videodensitometric methods. *Invest Radiol* 13:46-56, 1978
6. SOM P, YONEKURA Y, OSTER ZH, et al: Quantitative autoradiography with radiopharmaceuticals. Part 2: Applications in radiopharmaceutical research. *J Nucl Med* 24: 238-244, 1983
7. BIBERMAN LM: *Perception of Displayed Information.* Plenum Press, New York, 1973, Chapter 2
8. HALL E: *Computer Image Processing and Recognition*, Academic Press, New York, 1979, pp 166-173
9. LIGHT DL, BROWN D, COLVOCORESSES AP, et al: Satellite photogammetry. In *Manual of Photogrammetry.* American Society of Photogrammetry, 1980, pp 883-977
10. GOOCHEE C, RASBAND W, SOKOLOFF L: Computerized densitometry and color coding of (^{14}C) deoxyglucose autoradiographs. *Ann Neurol* 7:359-370, 1980
11. IRONS RD, GROSS EA: Standardization and calibration of whole-body autoradiography for routine semiquantitative analysis of the distribution of ^{14}C-labeled compounds in animal tissues. *Toxicol Appl Pharmacol* 59:250-256, 1981